PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	THE EFFECT OF POLYCYSTIC OVARY SYNDROME ON CARDIAC AUTONOMIC FUNCTION AT A LATE FERTILE AGE: A PROSPECTIVE NORTHERN FINLAND 1966 BIRTH COHORT STUDY
AUTHORS	Ollila, Meri-Maija; Kiviniemi, Antti; Stener-Victorin, Elisabet; Tulppo, Mikko; Puukka, Katri; Tapanainen, Juha; Franks, Stephen; Morin-Papunen, Laure; Piltonen, Terhi

VERSION 1 – REVIEW

REVIEWER	Gavin Lambert Swinburne University of Technology, Australia
REVIEW RETURNED	03-Sep-2019

GENERAL COMMENTS	Ollila and colleagues examined cardiac autonomic function in women with polycystic ovary syndrome (PCOS). While previous reports have undertaken similar investigations in younger women with PCOS this investigation focussed on women in their late reproductive years. The authors documented reduced parasympathetic function, as indicated by lower heart rate variability (HRV) and baroreflex sensitivity in women with PCOS, with the reduced HRV being associated with blood pressure, insulin resistance and plasma triglycerides rather than PCOS, BMI or free androgen index. Page 4 lines 75-80, the authors seem to be referring to various measures of sympathetic activity yet they finish by concluding that HRV is an effective and established measure. Whilst HRV does provide an index of parasympathetic activity evidence indicating a link between HRV and sympathetic activity is less persuasive. Although measures of HRV have been shown to be predictive of total cardiac mortality after myocardial infarction (La Rovere et al Lancet 1998) it is important to note that heart rate variability provides an index largely of vagal activity and does not provide a reliable index of cardiac sympathetic activity (Baumert et al Am J Physiol Heart Circ Physiol 2009; Martelli et al Am J Physiol Heart Circ Physiol, 2014). It may be worth reviewing and rewording this section. Heart rate variability was assessed at different locations, were there any differences between locations? Heart rate variability and baroreflex sensitivity were determined from 150 second recordings, approximately 170-200 heart beats. Is this sufficient recording period to obtain an accurate determination (please refer to guidelines for assessment of HRV). Could this be an additional limitation of the study? It is interesting that there seemed proportionately more women with PCOS using beta blockers (13.3 v 7.7%). Was this difference significant? Although the authors have justifiably excluded these

	women from the main analysis, was there any difference between the various measures in these women (both HRV measures and also metabolic measures given previous studies documenting metabolic side effects of beta blocker use). Page 10, lines 228-233, the authors use the term “normal weight”, would lean, overweight or obese be more accurate given that the majority of the population are tending to be overweight or obese? Were the authors able to determine weight gain between age 31 and 46? Whilst not necessarily the focus of the present report it would be intriguing to explore possible predictors of weight gain. Was BMI at age 14 associated with BMI at later ages? Discussion, lines 259-285, whilst interesting the focus seems to be often on sympathetic activity which was not assessed in the manuscript under review. Suggest subtle rewording. Similarly, lines 286-298. The authors cite reference 28 to demonstrate regionalisation of sympathetic outflows in obesity. This perhaps should be Vaz et al (Circulation 1997; 96:3425-3429) rather than the review of Lansdown and Rees. Please review author list for ref 14 For referencing increased sympathetic activity in women with PCOS (line 75) please also include ref 24 (Sverrisdottir et al) in addition to ref 13.

REVIEWER	Ingrid Tonhajzerova, prof., MD., PhD. Department of Physiology, Jessenius Faculty of Medicine in Martine, Comenius University in Bratislava, 036 01 Martin, Slovakia
REVIEW RETURNED	09-Sep-2019

GENERAL COMMENTS	The study regarding cardiovascular regulation with metabolic status interaction in PCOS at a late fertile age. The findings are interesting, however, I have several comments: 1. Five-minute segments (at least 300 RR intervals) are recommended for short-term HRV spectral analysis (Task Force, 1996). What was the reason for using of 150 seconds of recording in a seated and standing position? 2. For LF-SBP analysis, in humans, the frequency band from 0.075-0.15 Hz is recommended (Stauss, 2007). It should be correct the SBP frequency analysis at low-frequency band for correct interpretation of sympathetic modulation of vascular tone. 3. The interpretation of physiological mechanisms underlying LF-, HF-HRV, BRS, LF-SBP is missing. 4. It would be interesting to divide women into „overweight“ and „obese“ groups. 5. The part Discussion should be corrected. The short-term HRV is determined mainly parasympathetic regulation, therefore, the conclusion regarding sympathetic regulation is questionable. In this context, the interpretation of BPV analysis (sympathetic vascular regulation) is missing. Similarly, the BRS as heart rate and blood pressure reflex control should be discussed. Minor comments: „oligoamenorrhea“... should be „oligomenorrhea“ The method for blood pressure measurement should be included (auscultatory? oscillometric?)

VERSION 1 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1

Reviewer Name: Gavin Lambert

Institution and Country: Swinburne University of Technology, Australia

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

Ollila and colleagues examined cardiac autonomic function in women with polycystic ovary syndrome (PCOS). While previous reports have undertaken similar investigations in younger women with PCOS this investigation focussed on women in their late reproductive years. The authors documented reduced parasympathetic function, as indicated by lower heart rate variability (HRV) and baroreflex sensitivity in women with PCOS, with the reduced HRV being associated with blood pressure, insulin resistance and plasma triglycerides rather than PCOS, BMI or free androgen index.

1. **Page 4 lines 75-80, the authors seem to be referring to various measures of sympathetic activity yet they finish by concluding that HRV is an effective and established measure. Whilst HRV does provide an index of parasympathetic activity evidence indicating a link between HRV and sympathetic activity is less persuasive. Although measures of HRV have been shown to be predictive of total cardiac mortality after myocardial infarction (La Rovere et al Lancet 1998) it is important to note that heart rate variability provides an index largely of vagal activity and does not provide a reliable index of cardiac sympathetic activity (Baumert et al Am J Physiol Heart Circ Physiol 2009; Martelli et al Am J Physiol Heart Circ Physiol, 2014). It may be worth reviewing and rewording this section.**

   Response: We thank the reviewer for the comment, and we have now edited the lines 70–75 in page 4 to highlight that HRV mainly describes parasympathetic activity.

2. **Heart rate variability was assessed at different locations, were there any differences between locations?**

   Response: The protocol and the equipment's for the HRV assessment were exactly the same in all locations.

3. **Heart rate variability and baroreflex sensitivity were determined from 150 second recordings, approximately 170-200 heart beats. Is this sufficient recording period to obtain an accurate determination (please refer to guidelines for assessment of HRV). Could this be an additional limitation of the study?**

   Response: According to the HRV guideline (Heart Rate Variability - Standards of Measurement, Physiological Interpretation, and Clinical Use. Circulation 1996;93:1043–1065) "recording of approximately 1 minute is needed to assess the HF components of HRV, while approximately 2 minutes are needed to address the LF component. To standardize different studies investigating short-term HRV, 5-minute recordings of a stationary system are preferred unless the nature of the study dictates another design." Therefore, the 150 second i.e. 2.5-minute recordings used in the present study should be reliable. A longer recording period would have been more favorable, but due to logistical reasons and large number of subjects to measure,

we had to limit the time. Moreover, a recent study suggests that even a 60 second recording is sufficient for rMSSD assessment (Flatt and Esco, Clin Physiol Funct Imaging, 2016;36:331–336). We have now added text regarding this issue to the Discussion (Page 15, Lines 338–340)

4. **It is interesting that there seemed proportionately more women with PCOS using beta blockers (13.3 v 7.7%). Was this difference significant? Although the authors have justifiably excluded these women from the main analysis, was there any difference between the various measures in these women (both HRV measures and also metabolic measures given previous studies documenting metabolic side effects of beta blocker use).**

   Response: We thank the reviewer for this insightful comment. Indeed, women with PCOS significantly more often used beta-blockers (*P*=0.009). We have now added this *P*-value to the Page 8, Line 185. We also invite the Reviewer to read our previous publication on blood pressure and related medication in women with PCOS (Ollila *et al.* JCEM 2019).

   In our population, the women using beta-blockers had significantly higher systolic blood pressure (*P*<0.001), diastolic blood pressure (*P*<0.001), BMI (*P*<0.001), FAI (*P*=0.005), fasting glucose (*P*<0.001), fasting insulin (*P*<0.001), HbA1c (p<0.001), low-density lipoprotein (*P*<0.001) and triglycerides (p<0.001) as well as significantly lower serum level of sex-hormone binding globulin (*P*=0.005) and high-density lipoprotein (*P*<0.001), compared with the non-users, whereas serum levels of testosterone or total cholesterol did not significantly differ.

   As regards of the HRV parameters, the women using beta-blockers had significantly lower rMSSD (*P*=0.024), LF (*P*=0.024), HF (*P*=0.007), LF-HF ratio (*P*=0.007), α (*P*=0.004), and BRS (*P*=0.004) compared to the non-users, whereas HR and LF_{SBP} did not significantly differ between these two groups.

5. **Page 10, lines 228-233, the authors use the term "normal weight", would lean, overweight or obese be more accurate given that the majority of the population are tending to be overweight or obese?**

   Response: We have now changed the term "normal weight" to "lean", as suggested through the manuscript.

6. **Were the authors able to determine weight gain between age 31 and 46? Whilst not necessarily the focus of the present report it would be intriguing to explore possible predictors of weight gain. Was BMI at age 14 associated with BMI at later ages?**

   Response: We thank the reviewer for raising up this interesting issue. In fact, we have previously investigated the weight development of women with PCOS in this cohort (Ollila *et al*. JCEM 2016). We found that the women with PCOS and the control women had comparable weigh gain between ages of 31 and 46, whereas women with PCOS experienced significantly higher weight gain between ages of 14 and 31. BMIs at age 14, 31 and 46 were all significantly correlated, and PCOS was significantly associated with BMIs at ages 14, 31 and 46.

7. **Discussion, lines 259-285, whilst interesting the focus seems to be often on sympathetic activity which was not assessed in the manuscript under review. Suggest subtle rewording. Similarly, lines 286-298. The authors cite reference 28 to demonstrate regionalisation of sympathetic outflows in obesity. This perhaps should be Vaz et al (Circulation 1997; 96:3425-3429) rather than the review of Lansdown and Rees.**

   Response: We have now carefully edited and reworded the whole manuscript to clarify this issue. We have now changed the reference 28 to Vaz et al., as suggested.

   Please review author list for ref 14

Response: We have now corrected the reference to the correct form.

For referencing increased sympathetic activity in women with PCOS (line 75) please also include ref 24 (Sverrisdottir et al) in addition to ref 13.

Response: We have now added the reference to Sverrisdottir's work as suggested.

Reviewer: 2

Reviewer Name: Ingrid Tonhajzerova, prof., MD., PhD.

Institution and Country: Department of Physiology, Jessenius Faculty of Medicine in Martine, Comenius University in Bratislava, 036 01 Martin, Slovakia

Please state any competing interests or state 'None declared': None declared

Please leave your comments for the authors below

The study regarding cardiovascular regulation with metabolic status interaction in PCOS at a late fertile age. The findings are interesting; however, I have several comments:

1. **Five-minute segments (at least 300 RR intervals) are recommended for short-term HRV spectral analysis (Task Force, 1996). What was the reason for using of 150 seconds of recording in a seated and standing position?**

   Response: According to the HRV guideline (Heart Rate Variability - Standards of Measurement, Physiological Interpretation, and Clinical Use. Circulation 1996;93:1043–1065) "recording of approximately 1 minute is needed to assess the HF components of HRV, while approximately 2 minutes are needed to address the LF component. To standardize different studies investigating short-term HRV, 5-minute recordings of a stationary system are preferred unless the nature of the study dictates another design." Therefore, the 150 second i.e. 2.5-minute recordings used in the present study should be reliable. A longer recording period would have been more favorable, but due to logistical reasons and large number of subjects to measure, we had to limit the time. Moreover, a recent study suggests that even a 60 second recording is sufficient for rMSSD assessment (Flatt and Esco, Clin Physiol Funct Imaging, 2016;36:331–336). We have now added text regarding this issue to the Discussion (Page 15, Lines 338–340)

2. **For LF-SBP analysis, in humans, the frequency band from 0.075-0.15 Hz is recommended (Stauss, 2007). It should be correct the SBP frequency analysis at low-frequency band for correct interpretation of sympathetic modulation of vascular tone.**

   Response: We thank the reviewer for this comment. The frequency width of 0.04-0.15 Hz was needed for the calculation of BRS and the same frequency was used for LF-SBP. We concur that suggested frequency band (0.075-0.15 Hz) has some justification while lower limit of 0.04 Hz is also widely used (Malliani *et al.* Circulation 1991, Kiviniemi *et al*. Exp Physiol 2011). The frequency of LF oscillation varies a lot (Malliani *et al*. Circulation 1991, Kiviniemi *et al*. Exp Physiol 2011), also in relation to sympathetic effect (Kiviniemi *et al.* Exp Physiol 2011, Kiviniemi *et al.* Aut Neurosci 2010). It is therefore reasonable to use a wider spectral frequency band. Taken together, the main point is that the prevalent LF oscillation of blood pressure around 0.1 Hz is covered here. A modification of LF band for SBP spectral analysis would cause only subtle changes in the results that are, in any case, limited by the same methodological limitations. We have now underscored these limitations (Page 15, Lines 321–326).

3. **The interpretation of physiological mechanisms underlying LF-, HF-HRV, BRS, LF-SBP is missing.**

Response: We have now added text concerning this issue to Page 7, Lines 147–148 and Lines 158–163.

4. **It would be interesting to divide women into „overweight“ and „obese“ groups.**

   Response: We have now divided the population to the overweight and obese groups, as suggested. We found that the overweight women with PCOS (n=55) had significantly lower rMSSD (2.54±0.6 *vs*. 2.90±0.6, *P*=0.006), LF (4.91±0.9 *vs.* 5.52±0.9, *P*=0.034) and HF (4.30±1.2 *vs*. 5.00±1.2, *P*=0.010) compared to the overweight control women (n=328), whereas HR (80.0±11.2 *vs*. 75.8±9.6, *P*=0.064), LF/HF-ratio (0.61±0.9 *vs*. 0.51±0.9, *P*=0.187), LF_{SBP} (1.51±0.8 *vs*. 1.80±0.8, *P*=0.087), BRS (1.67±0.4 *vs*. 1.81±0.5, *P*=0.182) and α (1.68±0.4 vs. 1.81±0.5, *P*=0.182) did not differ between these two groups.

   We did not find any significant differences between the obese PCOS and control women in any HRV parameters, but this might be due to lack of statistical power, as there were only 23 obese women with PCOS (data not shown).

   We have now added this information to the Result-section (Page 10, Lines 236–244).

5. **The part Discussion should be corrected. The short-term HRV is determined mainly parasympathetic regulation, therefore, the conclusion regarding sympathetic regulation is questionable. In this context, the interpretation of BPV analysis (sympathetic vascular regulation) is missing. Similarly, the BRS as heart rate and blood pressure reflex control should be discussed.**

   Response: We thank the reviewer for the comment, and we have now rewritten the discussion (Page 13: Lines 265–270, Page 14: Lines 297–298, 305–306, and Page 15: Lines 341–342), and also added discussion concerning BRS and LF_{SBP} (Pages 14–15, Lines 319–327).

   Minor comments:

   „oligoamenorrhea“... should be „oligomenorrhea“

   Response: We have now changed the term to be “oligomenorrhea/amenorrhea”.

   The method for blood pressure measurement should be included (auscultatory? oscillometric?)

   Response: We have now added to the text (Page 5, Lines 109–110), that blood pressure was measured by an automated, oscillometric blood pressure device with an appropriately sized cuff (Omron Digital Automatic Blood Pressure Monitor Model M10-IT; Omron, Kyoto, Japan.

VERSION 2 – REVIEW

REVIEWER	Gavin Lambert Iverson Health Innovation Research Institute, Swinburne University of Technology
REVIEW RETURNED	07-Oct-2019

GENERAL COMMENTS	The authors have addressed my comments. I have one minor suggestion regarding the terminology used, at times, regarding LF HRV as a marker of sympathetic activity. At times the authors use terminology stating that HRV provides an index of sympathetic

	activation (line 150, line 322 for example) but then state that it is not a reliable marker. My preference would be to not equivocate. The pertinent work in my opinion is the experimental work of Martelli (Martelli, D., A. Silvani, R.M. McAllen, et al. 2014. The low frequency power of heart rate variability is neither a measure of cardiac sympathetic tone nor of baroreflex sensitivity. Am J Physiol Heart Circ Physiol. 307: H1005-1012) and the clinical observations of Baumert (Baumert, M., G.W. Lambert, T. Dawood, et al. 2009. Short-term heart rate variability and cardiac norepinephrine spillover in patients with depression and panic disorder. Am J Physiol Heart Circ Physiol. 297: H674-679).

VERSION 2 – AUTHOR RESPONSE

Reviewer(s)' Comments to Author:

Reviewer: 1
Reviewer Name: Gavin Lambert

Institution and Country: Iverson Health Innovation Research Institute, Swinburne University of Technology

Please state any competing interests or state 'None declared': None declared
Please leave your comments for the authors below

The authors have addressed my comments. I have one minor suggestion regarding the terminology used, at times, regarding LF HRV as a marker of sympathetic activity. At times the authors use terminology stating that HRV provides an index of sympathetic activation (line 150, line 322 for example) but then state that it is not a reliable marker. My preference would be to not equivocate. The pertinent work in my opinion is the experimental work of Martelli (Martelli, D., A. Silvani, R.M. McAllen, et al. 2014. The low frequency power of heart rate variability is neither a measure of cardiac sympathetic tone nor of baroreflex sensitivity. Am J Physiol Heart Circ Physiol. 307: H1005-1012) and the clinical observations of Baumert (Baumert, M., G.W. Lambert, T. Dawood, et al. 2009. Short-term heart rate variability and cardiac norepinephrine spillover in patients with depression and panic disorder. Am J Physiol Heart Circ Physiol. 297: H674-679).

Response: We agree with the Reviewer and we have now edited the manuscript (Page 7, Lines 148 – 153) to clarify the background of the low frequency oscillation of the HRV. As previous studies have used LF and LF/HF-ratio, we also included these parameters to enable the comparison between our work and previous reports.

Another mentioned part of the text in the reviewer's comment above (previous line 322, currently 324 – 325) focuses on the low frequency oscillation of the systolic blood pressure and has already been edited according to the comments of the Reviewer 2 (in the first revision round).